

# Antitumor efficacy of Aqueous Extract of *Salenostemma argel* Leaves and/or $\gamma$ -radiation exposure against Ehrlich Carcinoma in Swiss Albino Mice

N. Hanafi and S.Z. Mansour

Radiation Biology Dept., National Centre for Radiation Research and Technology (NCRRT), P.O.Box:29 Nasr City, EGYPT

[Nhanafi58@yahoo.com](mailto:Nhanafi58@yahoo.com) and [szmansour@yahoo.com](mailto:szmansour@yahoo.com)

## ABSTRACT:

**Background-** The *Salenostemma argel* (SA) leaves aqueous extract either alone or combined with 6 Gy of  $\gamma$ - radiation exposure were tested to evaluate thier antitumor effect against Ehrlich carcinoma (EC) bearing mice. **Methods and Results-** SA extract was gavages at the doses of 15 mg/kg body weight / day x 3/ week for 3 weeks. SA gavages to experimental animals 24 hr and 7 days after tumor inoculation (ATI).  $\gamma$ - radiation exposure was done 7 days ATI as a single dose. In the present study aqueous extract of SA chemosensitivity, growth of transplantable murine tumor, histopathological examination, apoptotic and necrotic detection and biochemical parameters such as lipid peroxidation (MDA), glutathione content (GSH), superoxide dismutase (SOD), and catalase (CAT) activities for the murine tumor and liver tissues were done. SA activates tumor cell death after two weeks. SA significantly decrease tumor volume either alone or combined with  $\gamma$ - irradiation. SA recorded high and wide zones of apoptotic tumor cells either alone or combined with  $\gamma$ - irradiation. SA and  $\gamma$ -irradiation resultant a non significant change in MDA and a non significant change in GSH level and CAT activity in tumor tissue either alone or combined with  $\gamma$ - irradiation. SA ameliorates MDA and significantly ameliorates GSH level and CAT and SOD activity in liver tissue of EC-bearing mice. Histopathologically SA and/or  $\gamma$ -irradiation represented large aria of apoptosis, hydropic degeneration and nuclear debris in tumor tissue section. However in liver tissue SA treatment represent increase in Kupffer cells and decrease of inflammatory cells. SA and  $\gamma$ -irradiation represented normal appearance of liver tissue section. **Conclusion -** SA and/ or  $\gamma$ -irradiation represent antitumor activities.

**Keywords:** *Salenostemma argel* (SA),  $\gamma$ -irradiation, Ehrlich carcinoma (EC).

## INTRODUCTION

Cancer has become an important topic in medicine since it is a major cause of death in both the developed and developing countries and it is now only secondary to that of myocardial infarction [1]. Modern surgery has significantly reduced the cancer mortality. Also the use of additional treatment such as radiotherapy and chemotherapy has resulted in no more than 5% reduction in the number of deaths [2]. Many plants and animal extracts have shown various biological activities like immunopotentiating and antitumor activities. Therefore, there is a continuing search for better control and preventive methods in order to reduce cancer mortality and related side effects. Many investigations are now being carried out to discover naturally occurring compounds, which can suppress or prevent the process of carcinogenesis [3].

Radiation therapy is considered to be one of the most popular and important tools to cure cancer. The killing action of ionizing radiation (X-rays,  $\gamma$ -rays) is mainly mediated through the free radicals generated from the radiolytic decomposition of cellular water. This free radical species, when interact with critical targets like DNA and membranes bring about irreversible damage leading to cell death. Cell survival and adaptation to an environment containing ionizing radiation could mainly depend on the ability of cells to maintain optimal function in response to free radical induced damage at the biochemical level [4]. However radiation therapy of cancer as the elevated antioxidant status of irradiated tumors is likely to limit the effectiveness of radiation dose and adversely affect the

therapeutic gain [4]. Some serious implications as the increased radiation-damage of the distant normal organs (due to tumor burden) are likely to adversely affect the therapeutic gain [6].

*Salenostemma argel* (SA) is a shrub occurring in wiled state in Egypt, and Sudan. The herb of the plant locally called El Hargal [7]. The natives prepare decoctions from the leaves and branches for treatment of various colics and pains [7]. The Ehrlich ascites tumor cell is a spontaneous murine mammary adenocarcinoma adapted to ascites form [8] and carried in outbred mice by serial intraperitoneal (i.p.) passage.

For developing countries the use of endogenous medicinal plants as cures against cancers is attractive. This study was therefore designed to study the effect of *Salenostemma argel* (SA) leaves (aqueous extract) either alone or combined with 6 Gy of  $\gamma$ - radiation exposure to evaluate its antitumor activity against Ehrlich carcinoma (EC) bearing mice.

## MATERIALS AND METHODS

Adult male Swiss albino mice weighing 22-25g purchased from the breeding unit of the Egyptian Organization for Biological Products and Vaccines (Cairo) were used in this study. The animals were maintained on a commercial standard pellet diet and tap water ad libitum.

### *Radiation facility:*

Whole body gamma irradiation of animals was performed using a Canadian <sup>137</sup>Cs Gamma Cell-40 at the National Center for Radiation Research and

Technology (NCRRT), Cairo, Egypt; at a dose rate of 0.61 Gy/min. Rats were exposed to 6Gy whole body  $\gamma$ -radiation delivered as a single dose.

### ***Tumor Transplantation***

A cell line of Ehrlich Ascites Carcinoma (EAC) was used in this study. The parent line was supplied from Egyptian National Cancer Institute (NCI), Cairo University. The tumor line was maintained in the experimental female Swiss albino mice by weekly intraperitoneal injection of 2.5 millions cells per mouse. The EAC cells were counted before intraperitoneal injection using the bright line haemocytometer and dilution was done using physiological sterile saline solution. The desired numbers of cells were injected in a volume of 0.2 ml. To assess Ehrlich Solid Tumor, 0.2 ml EAC cells ( $2.5 \times 10^6$  cells/mouse) were inoculated intramuscularly in the right thigh of the lower limb of female mouse.

### ***Extraction of Salenostemma argel (SA) leaves.***

The leaves of SA were free from the other parts of the plant and crushed by hand, then 10 gm of the leaves were extracted with 100 ml of distilled water in a water bath for 90 min., then the volume was adjusted to 100 ml by passing hot water through the residue. The extract was administered to experimental animals at the doses of 15 mg/kg body weight /day  $\times$  3/ week.

### ***Experimental design***

Aqueous extract of SA either alone or combined with 6 Gy of  $\gamma$ - radiation exposure were tested to evaluate its antitumor activity against Ehrlich carcinoma (EC) bearing mice. Salenostemma argel (SA) was administered to experimental animals 24 hr and 7 days after tumor inoculation (ATI).  $\gamma$ - radiation exposure was done 7 days ATI. The animals were randomly divided into seven sets; Set 1: Normal mice; Set 2: Mice bearing EC. Set 3: Mice bearing EC exposed to  $\gamma$ - radiation 7 days ATI; Set 4: SA treated mice bearing EC 24 hours ATI; Set 5: SA treated mice bearing EC 7 days ATI Set 6: SA treated mice bearing EC 24 hours ATI and exposed to  $\gamma$ - radiation 7 days ATI. Set 7: SA treated mice bearing EC and exposed to  $\gamma$ - radiation 7 days ATI. 24 hrs after the last treatment the mice were sacrificed.

### ***Chemosensitivity of SA***

To study the Chemosensitivity of SA extract on Ehrlich ascites carcinoma cells (EACC) 10 animals of female Swiss albino mice were transplanted with EACC and each mouse was day per day forced to ingest orally via a stomach tube about 0.2 ml of hot water SA extract. After one week samples of EACC cells were taken and the number of EACC was calculated for each animal in each week.

### ***Tumor volume monitoring***

The growth of tumor was monitored throughout the experiment with the tumor volume being measured regularly twice or thrice weekly using Vernier calipers and represented in terms of tumor volume. The tumor volume was estimated using the following formula: Tumor volume (mm<sup>3</sup>) =  $4(A/2) (B/2)^2 \times 0.25$  A.B<sup>2</sup>, where A is the major axis and B is the minor axis [9]. The mean tumor volume with the corresponding standard error was calculated in each experimental group. 3 weeks ATI experiment was terminated. At the termination of the experiment, the animals were sacrificed.

### ***Pathological examination.***

At the time of sacrifice, the tumours and livers were excised from the animals. Samples from each of liver and tumour tissues were fixed in 10% formalin and embedded in paraffin. Sections of tissues were stained with Hematoxylin and eosin.

### ***Apoptosis and Necrosis examination.***

For Apoptosis and Necrosis examination according to Bank [10] fluorescence microscopy was used. Deparaffinization was done by immersing tumor sample slides in 3 changes of xylene for 5 minutes each followed by washing in graded alcohol as follows: 100%, 95%, 80%, and 50% for re-hydration. Two changes for 3 minutes in each alcohol concentration were done. Then, they were rinsed in 3 changes of PBS. Afterwards, slides were directly incubated in (5  $\mu$ g/ml of propidium iodide and 50  $\mu$ g/ml of acridine orange in phosphate-buffered saline) in dark for 1 h at room temperature.

### ***The preparation of tissue samples***

After cervical decapitation of mice, liver and tumor tissues were sampled and washed with 0.9% NaCl solution and stored at -20°C till biochemical analysis.

### ***Assay of reduced glutathione (GSH)***

GSH concentration was measured by the method of Moron et al. [11]. Briefly, proteins were precipitated by 25% TCA, centrifuged and the supernatant was collected. The supernatant was mixed with 0.2 M sodium phosphate buffer pH 8.0 and 0.06 mM DTNB and incubated for 10 min at room temperature. The absorbance of the sample/s was read against the blank at 412 nm in a UV-Visible double beam spectrophotometer and the GSH concentration was calculated from the standard curve.

### ***Assay of malondialdehyde (MDA)***

MDA was measured by the method of Beutge and Aust [12]. Briefly, the homogenate was mixed with TCA-TBA-HCl and heated for 15 min in a boiling-water bath. After centrifugation the absorbance was

recorded at 535 nm using a UV-Visible double-beam spectrophotometer.

## Assay of SOD

The activity of SOD in tissue was assayed by the method of Kakkar et al., [13]. The assay mixture contained 1.2 mL sodium pyrophosphate buffer (pH 8.3, 0.025 mol/L), 0.1 mL phenazine methosulphate (186 mmol/L), 0.3 mL nitroblue tetrazolium (300 mmol/L), 0.2 mL NADH (780 mmol/L) and diluted enzyme preparation and water in a total volume of 3 mL. After incubation at 30°C for 90 sec, the reaction was terminated by the addition of 1.0 mL of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4.0 mL n-butanol. The color intensity in the butanol layer was measured at 560 nm against n-butanol.

## Assay of Catalase (CAT)

Catalase was assayed according to the method of Maehly and Chance [14]. The estimation was done spectrophotometrically following the decrease in absorbance at 230 nm. The tissue was homogenized in M/150 phosphate buffer (pH 7.0) at 4°C and centrifuged at 5000 rpm. The reaction mixture contained 0.01 M phosphate buffer (pH 7.0), 2 mM H<sub>2</sub>O<sub>2</sub> and the enzyme extract. The specific activity of catalase is expressed in terms of units/mg protein. A unit is defined as the velocity constant per second.

## Statistical Analysis

Statistical analysis for obtained results was carried out with the aid of the SPSS computer software program.

## RESULTS

### Chemosensitivity of SE extract

When SA extracts were administrated to mice the numbers of Ehrlich ascite carcinoma cells (EACCs) in tumor-bearing mice were significantly reduced. A non significant change in viable cell no. was recorded after one week. However SA extracts appear to activate EACC tumor cell death possibly after two weeks.

**Table 1. Effect of the extracts on the number of viable cells in transplanted mice**

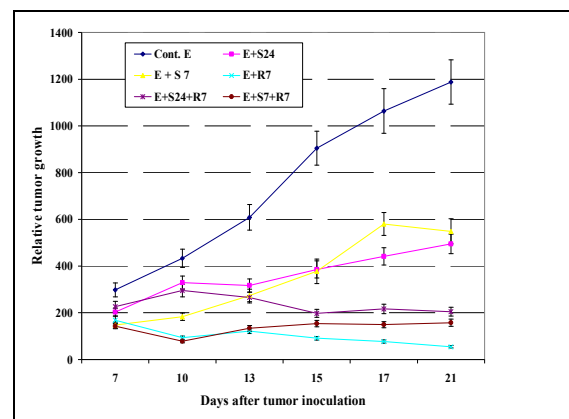
Groups	One week (- ×106)	Two weeks (- ×106)
Cont. E	83 ± 3.90	479 ± 22.51
E+SA	81 ± 2.92	47 ± 1.69

Each value represents the mean ± S.D and mean of three replicates.

### Tumor volume

Mice-bearing EC were gavages with SA either 24 hr. or 7 days of tumor cells inoculation and/or  $\gamma$ - radiation exposure up until day 21. Antitumor activity was assessed by time interval measurements of changes in TV during the experimental time course. Fig (1) shows that gavages of mice with SA either 24 hr or 7 days ATI or exposure to  $\gamma$ - radiation 7 days ATI caused a continuous delay of tumor volume recorded 317.000

±28.40 mm<sup>3</sup>, 274.200±27.00 mm<sup>3</sup> and 274.200 ±10.47 mm<sup>3</sup> respectively compared with control level 608.75 ± 54.70 mm<sup>3</sup> on the 13<sup>th</sup> day ATI. A more pronounced delay in tumor volume recorded when experimental animals gavages administrated with SA either 24 hr or 7 days ATI combined with exposure to 6 Gy of  $\gamma$ - radiation 7 days ATI. The tumor volume reached 204.850 ±18.85 mm<sup>3</sup> and 157.933 ±15.16 mm<sup>3</sup> respectively comparing to 1188.25 ± 95.00 control tumor volumes after 21 day of tumor inoculation.



**Fig. 1** Effect of SA and/or  $\gamma$ -radiation on tumor volume of Ehrlich carcinoma.

### Apoptotic and necrotic examination of the Ehrlich carcinoma

Apoptotic and necrotic examination of the Ehrlich carcinoma tumor under a fluorescent microscope showed that Ehrlich carcinoma represent viable tissue (green cells) with no zones of necrosis (orange cells) nor apoptosis (yellow cells) (fig2-1). Treatment of female mice bearing Ehrlich carcinoma tumor 24 h. ATI for 3 weeks with SA represented viable tissue (green cells) with no zones of necrosis (orange cells) but with high and wide zones apoptotic cells (yellow cells ↓) (fig2- 2&2a). Less effect were observed in tumor tissue section when experimental animals began gavages with SA on the 7<sup>th</sup> day ATI represent a viable tissue (green cells) with moderate sporadic apoptotic cells (yellow cells ▲) (fig2-3a) but with multiple vacuoles(↑)(fig2-3).

Exposure of the experimental animals to 6 Gy of  $\gamma$ - radiation on the 7<sup>th</sup> day ATI predict viable tissue observed as green cells with sporadic apoptotic yellow cells (fig.3- 4&4a) after 3 weeks from radiation exposure. In the tumors extirpated from animals treated with SA 24 h. ATI for 3 weeks and exposed to 6 Gy of  $\gamma$ - radiation on the 7<sup>th</sup> day ATI extensive high and wide zones of apoptotic cells (↑)with presence of some vacuoles (▲) was observed (fig.3- 5&5a). While in the group treated with SA on the 7<sup>th</sup> day ATI and exposed to 6 Gy of  $\gamma$ - radiation, no zones of necrosis (orange cells) were observed but with high and wide zones of apoptotic cells (curved arrows) (fig.3- 6&6a).

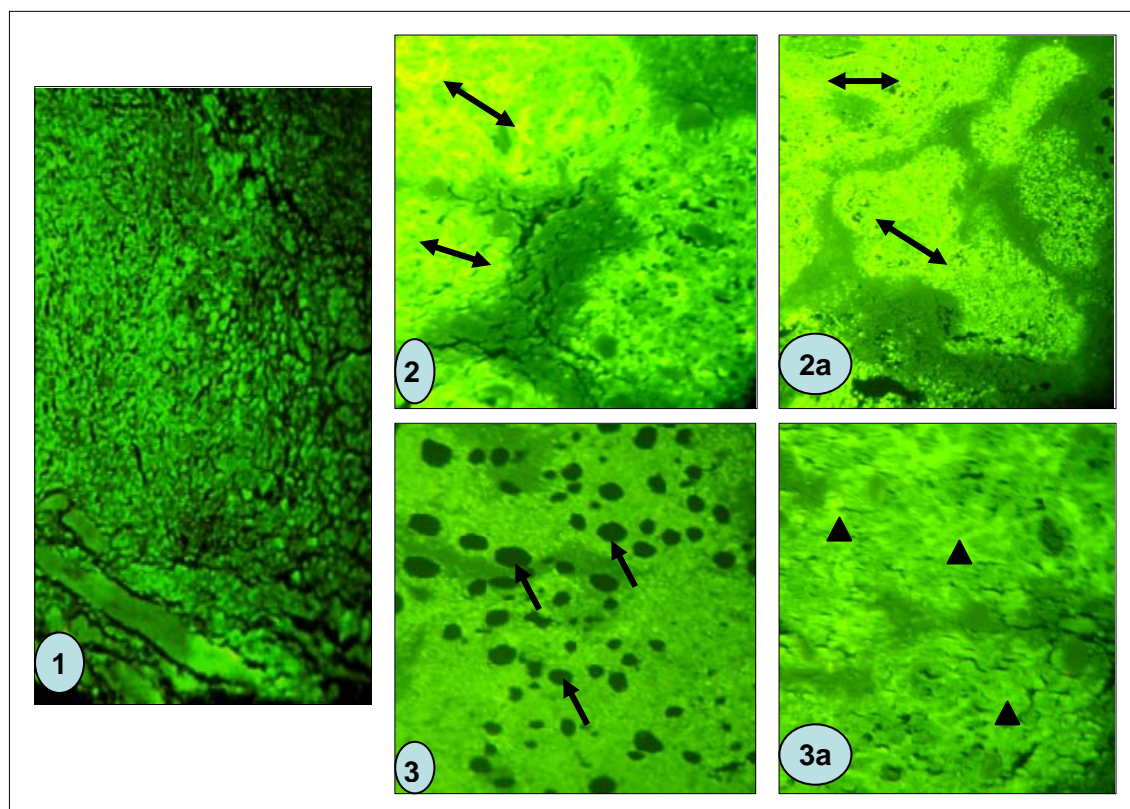


Fig.2 Fluorescent photomicrographs of sections in solid Ehrlich carcinoma (SEC). 1: Section in normal SEC. 2 & 2a: Section in SEC treated by SA 24 hr ATE. 3&3a: Sections in SEC treated by SA 7 days ATE.

Table2. Effect of SA and/or  $\gamma$  – radiation on antioxidant parameters of tumor tissue in mice bearing EC.

Groups	Lipid peroxides ( $\mu\text{g/g}$ tissue)	Reduced glutathione ( $\mu\text{g/g}$ tissue)	Catalase ( $\mu\text{mol/g}$ tissue)	Superoxide dismutase ( $\mu\text{g/g}$ tissue)
E	111.542 $\pm$ 4.86	67.703 $\pm$ 2.67	26.668 $\pm$ 2.70	4.708 $\pm$ 0.10
E+S 24h	102.575 $\pm$ 3.25	70.643 $\pm$ 2.98	25.643 $\pm$ 2.08	4.303 $\pm$ 0.15
E+ S 7 day	105.380 $\pm$ 2.24	71.335 $\pm$ 3.52	23.835 $\pm$ 1.75	4.715 $\pm$ 0.15
E+R 7day	145.928 $\pm$ 3.13 <sup>b</sup>	73.668 $\pm$ 1.92	21.018 $\pm$ 1.19	3.640 $\pm$ 0.10 <sup>b</sup>
E+S+R 24h	108.660 $\pm$ 2.37	73.750 $\pm$ 1.87	23.575 $\pm$ 1.78	4.228 $\pm$ 0.22 <sup>b</sup>
E+S+R 7day	117.018 $\pm$ 2.12	67.778 $\pm$ 1.92	25.728 $\pm$ 2.37	3.923 $\pm$ 0.12 <sup>b</sup>

Results are presented as the mean  $\pm$  SE.

<sup>b</sup> significant from Ehrlich group at  $p \leq 0.05$

### Effect of SA and/or $\gamma$ – radiation on antioxidant parameters

As shown in table (2) the levels of lipid peroxidation in tumor tissue were non significantly changed when experimental animals treated with SA either after 24 h. or 7 days of tumor inoculation compared to normal tumor level. However the exposure of the tumor bearing mice to 6 Gy  $\gamma$ -radiation 7 days ATI predict a significant increase ( $p \leq 0.05$ ) in lipid peroxides in tumor tissue 21 days ATI. Combined treatment of tumor bearing mice with SA and  $\gamma$ -radiation exposure resultant a non significant change in lipid peroxides in tumor tissue either for SA 7 days ATI or 24 hr. ATI. Gavage administration of SA 24 hr. ATI or 7 days ATI either

alone or combined with  $\gamma$ -radiation exposure predict a non significant change in GSH and CAT. However  $\gamma$ -radiation exposure either alone or combined with SA administration predict some ameliorative effect in SOD level compared to control tumor tissue level.

ROS formed in cancer tissues results in lipid peroxidation and subsequently to increase in malondialdehyde (MDA) level. Table 3 depicts the levels of MDA in liver tissue of experimental animals. In the present study, the levels of MDA were significantly ( $p < 0.05$ ) increased in EC bearing animals when compared with control animals. After treatment with SA ameliorative effect in liver tissue

was predicted either for 24 hr. or 7days ATI. Also exposure to 6 Gy  $\gamma$ -radiation 7 days ATI predict a significant decrease ( $p \leq 0.05$ ) in lipid peroxides in liver tissue compared to EC bearing mice. Combined treatments of EC bearing mice with SA 24 hr. ATI followed by exposure to 6 Gy  $\gamma$ -radiation 7 days ATI A significant decline in GSH, CAT and SOD levels in the livers of tumor-bearing animals was observed

compared to the normal control level. Also the exposure of the tumor bearing animals to 6 Gy of  $\gamma$ -radiation predicts the same effect. Gavages treatment of EC bears animals by SE either 24 hr. or 7days ATI either or combined with  $\gamma$ -radiation exposure 7days ATI resultant a significant amelioration in GSH, CAT and SOD levels in livers of EC bearing animals.

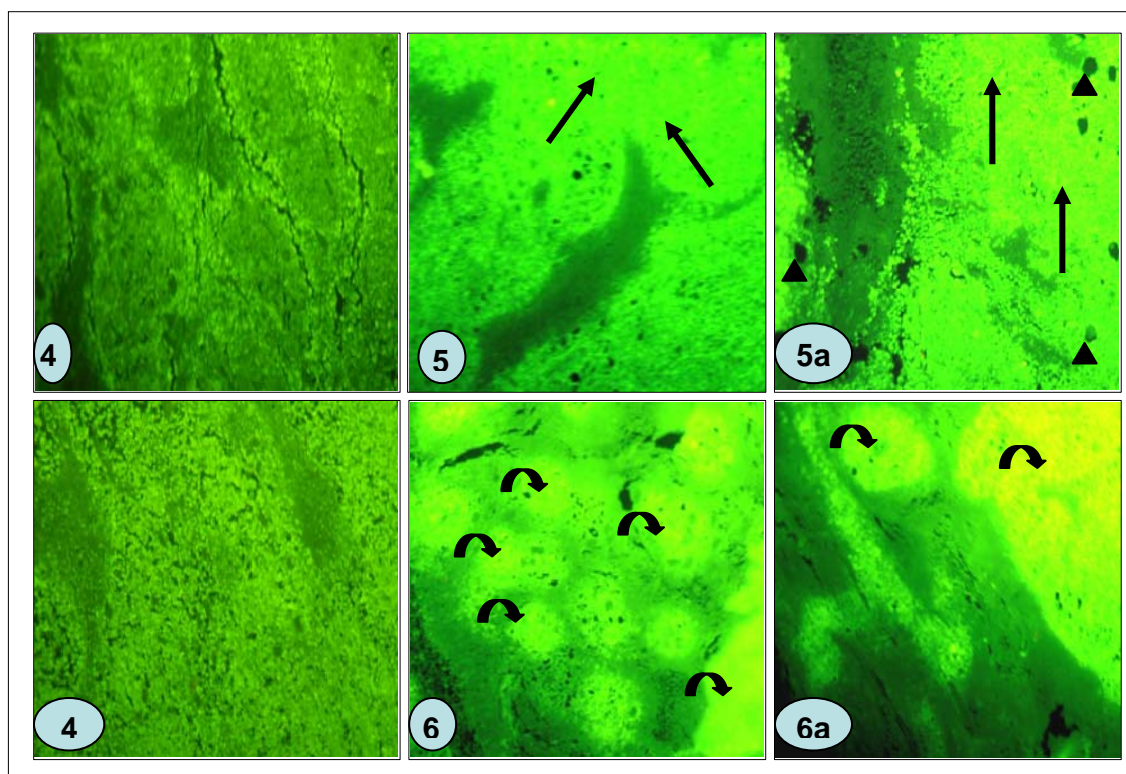


Fig.3 Fluorescent photomicrographs of sections in solid Ehrlich carcinoma (SEC). 4&4a: Section in SEC subjected to  $\gamma$ - radiation7 days ATE. 5&5a: Section in SEC treated by SA 24 hr ATE and subjected to  $\gamma$ - radiation7 days ATE. 6&6a: Sections in SEC treated by  $\gamma$ - radiation and SA 7 days ATE.

Table 3. Effect of SA and/or  $\gamma$ -radiation on antioxidant parameters of liver tissue in mice bearing EC.

Groups	Lipid peroxides ( $\mu\text{M}/\text{g}$ tissue)	Reduced glutathione ( $\text{mg}/\text{g}$ tissue)	Catalase ( $\mu\text{mol}/\text{g}$ tissue)	Superoxide dismutase ( $\mu\text{g}/\text{g}$ tissue)
Control	$126.657 \pm 2.55^b$	$23.735 \pm 1.32^b$	$62.125 \pm 1.14^b$	$531.223 \pm 8.61^b$
E	$173.983 \pm 3.94^a$	$15.613 \pm 2.01^a$	$41.555 \pm 1.22^a$	$322.403 \pm 3.84^a$
E+S 24h	$151.208 \pm 1.03^{ab}$	$19.970 \pm 1.01^{ab}$	$56.390 \pm 1.26^{ab}$	$389.095 \pm 5.59^{ab}$
E+S 7 day	$128.265 \pm 1.13^b$	$22.690 \pm 0.71^b$	$57.390 \pm 0.40^{ab}$	$426.638 \pm 7.89^{ab}$
E+R 7day	$145.485 \pm 4.54^{ab}$	$15.688 \pm 0.99^a$	$32.750 \pm 1.02^{ab}$	$352.638 \pm 3.69^{ab}$
E+S+R 24h	$152.728 \pm 1.25^{ab}$	$20.935 \pm 1.05^b$	$51.473 \pm 0.74^{ab}$	$403.740 \pm 1.05^{ab}$
E+S+R 7day	$128.155 \pm 1.41^b$	$19.163 \pm 0.66^{ab}$	$55.918 \pm 0.75^{ab}$	$438.305 \pm 6.11^{ab}$

Results are presented as the mean  $\pm$  SE.

a significant from control group at  $p \leq 0.05$

b significant from Ehrlich group at  $p \leq 0.05$

### Histopathological examination of the Ehrlich carcinoma

Histopathological examination of the Ehrlich carcinoma tumor under light microscope showed compact and aggregation of the tumor tissue cells spread within the muscular tissues. Ehrlich carcinoma tumor showed

groups of large, round and polygonal cells, with pleomorphic shapes, hyperchromatic nuclei and binucleation. Several degrees of cellular and nuclear pleomorphism were seen. Mitosis, muscle invasion and coagulation necrosis were also noticed (Fig.4 A). Treatment of female mice bearing Ehrlich carcinoma tumor 24 h. ATI for 3 weeks with SA represented large

aria of apoptosis ( $\downarrow$ ) hydropic degeneration (blocked arrow) and nuclear debris ( $\blacktriangle$ ) were also detected in tumor tissue section (Fig.4 B). Another effect was observed in tumor tissue section when experimental animals began gavages with SA on the 7<sup>th</sup> day ATI (Fig.4 C& Ca). Highly degenerative effect was detected in tumor tissue section extirpated from animals exposed to 6 Gy of  $\gamma$ - radiation on the 7<sup>th</sup> day ATI (Fig.5 D). Tumor tissue section represented great arias of apoptosis ( $\downarrow$ ) in addition of some pyknotic nuclei ( $\blacktriangle$ ). In the tumors extirpated from animals treated with SA 24 h. ATI for 3 weeks and exposed to 6 Gy of  $\gamma$ - radiation on the 7<sup>th</sup> day ATI extensive areas of apoptosis were observed ( $\downarrow$ ). In addition to the presence of remnant tumor cells contained pyknotic nuclei ( $\blacktriangle$ ) were detected (Fig.5 E & Ea). While in the group treated with SA on the 7<sup>th</sup> day ATI and exposed to 6 Gy of  $\gamma$ - radiation, apoptotic tumor cells ( $\uparrow$ ) and nuclear debris ( $\downarrow$ ) were observed (Fig.5F&Fa).

### Histological observations of liver tissue

The present results demonstrated the normal polyhedral hepatocytes with granular cytoplasm in liver tissue. Each cell has a centrally located nucleus with one or two nucleoli in addition to a number of chromatin particles (Fig.6-G). Liver of animals bearing EC (Fig.6-H) showed alterations as Kupffer cells

hyperplasia (curved arrow), intense ballooning degeneration of hepatocytes ( $\blacktriangle$ ), great aggregation of inflammatory cells filtration and Ehrlich tumor cells ( $\uparrow$ ). Treatment of the experimental animals with SA 24 h. ATI for 3 weeks (Fig.6-I) represented increase in Kupffer cells ( $\uparrow$ ), decrease in amount of inflammatory cells (curved arrow) and some liver steatosis was also detected. Also the beginning of treatment of the experimental animals with SA on 7th days ATI (Fig.6-J&Ja) represented less effect in which some aggregation of inflammatory cells filtration and Ehrlich tumor cells (curved arrows) were detected. Exposure of experimental animals bearing EC to 6 Gy of  $\gamma$ - radiation on the 7<sup>th</sup> day ATI the liver cells were filled with some cytoplasmic material and the tissue represent some vacuolisation ( $\uparrow$ ). The size of the nuclei was essentially the same as that of normal cells and cells with two nuclei were considerably fewer than in tumor bearing animal group (Fig.7-K). Normal appearance in liver tissue was detected when female mouse bearing Ehrlich carcinoma gavages with SA 24 h. ATI for 3 weeks and exposed to 6 Gy of  $\gamma$ - radiation on the 7<sup>th</sup> day ATI (Fig.7-L). While in the group treated with SA on the 7<sup>th</sup> day ATI and exposed to 6 Gy of  $\gamma$ - radiation, a well developed hepatic cord was detected in addition to presence of ballooning degeneration observed in some hepatocytes ( $\uparrow$ ) (Fig.7-M).

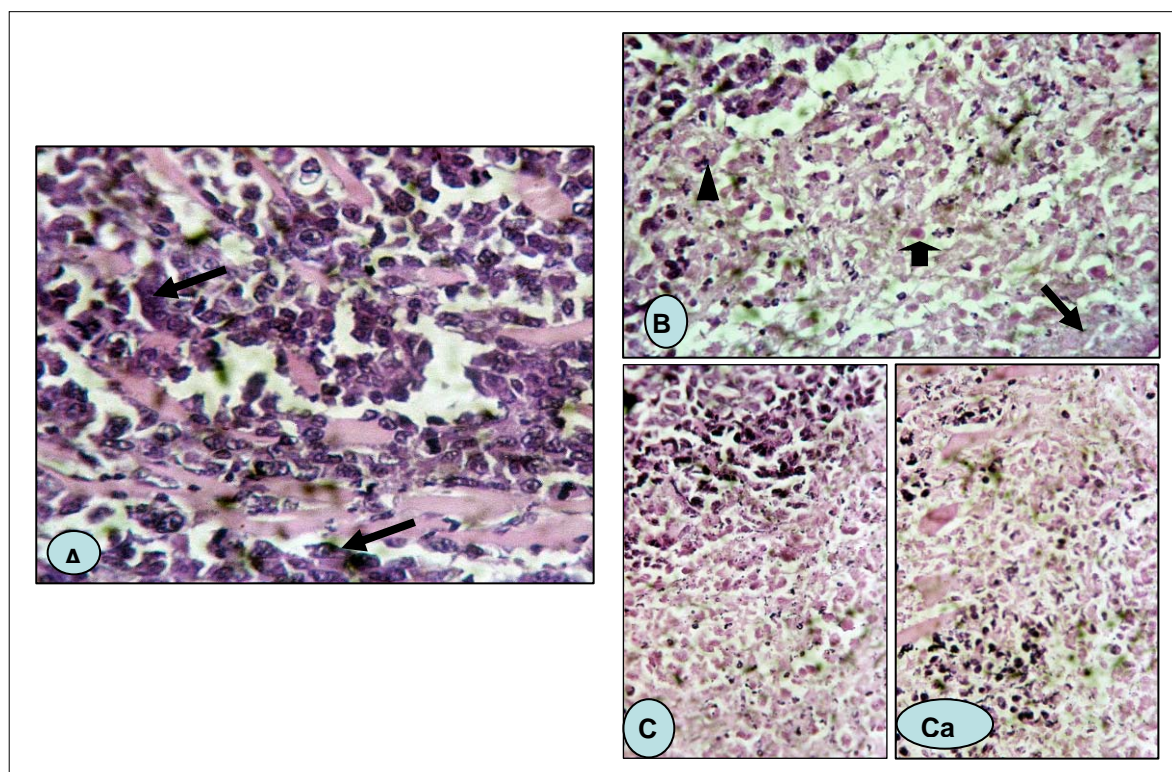


Fig.4 Photomicrographs of sections in solid Ehrlich carcinoma (SEC). A: Section in normal SEC. B: Section in SEC treated by SA 24 hr ATE. C&Ca: Sections in SEC treated by SA 7 days ATE. (H&E. X400)

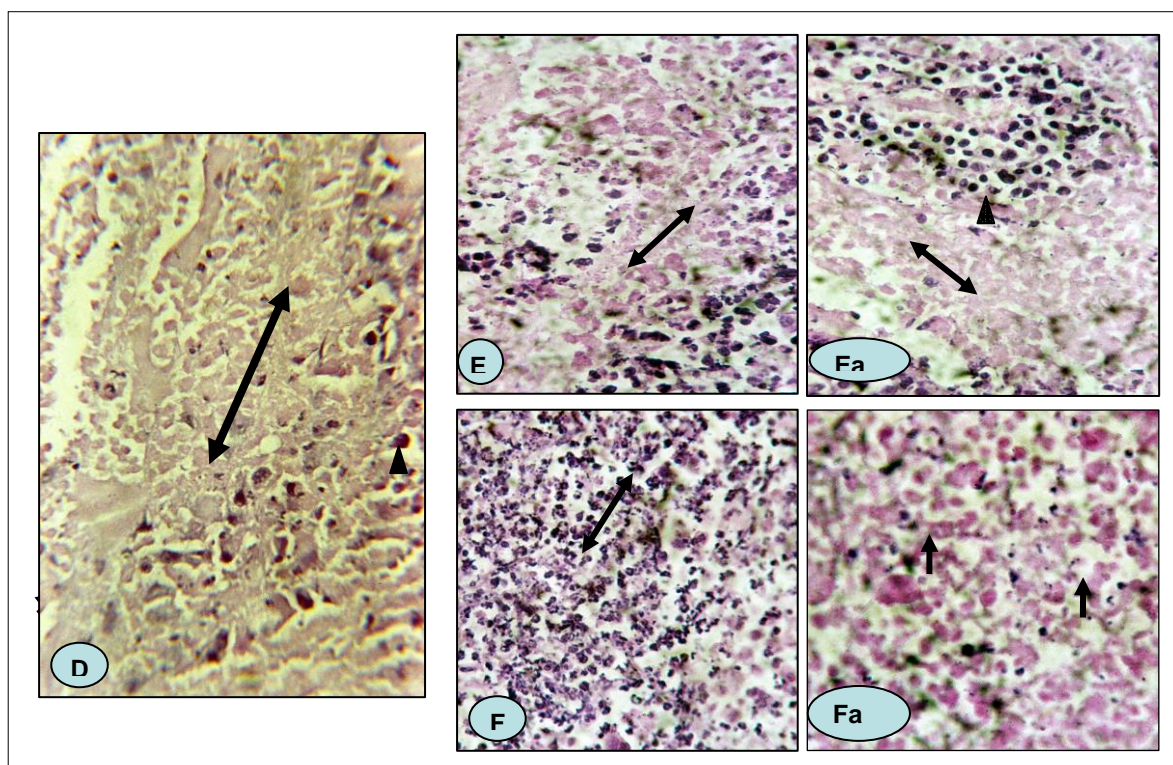


Fig.5 Photomicrographs of sections in solid Ehrlich carcinoma (SEC) in mice subjected to  $\gamma$ - radiation7 days ATE. **D**: Section in SEC subjected to  $\gamma$ - radiation7 days ATE. **E&Fa**: Section in SEC treated by SA 24 hr ATE and subjected to  $\gamma$ - radiation7 days ATE. **F&Fa**: Sections in SEC treated by  $\gamma$ - radiation and SA 7 days ATE. (H&E. X400)

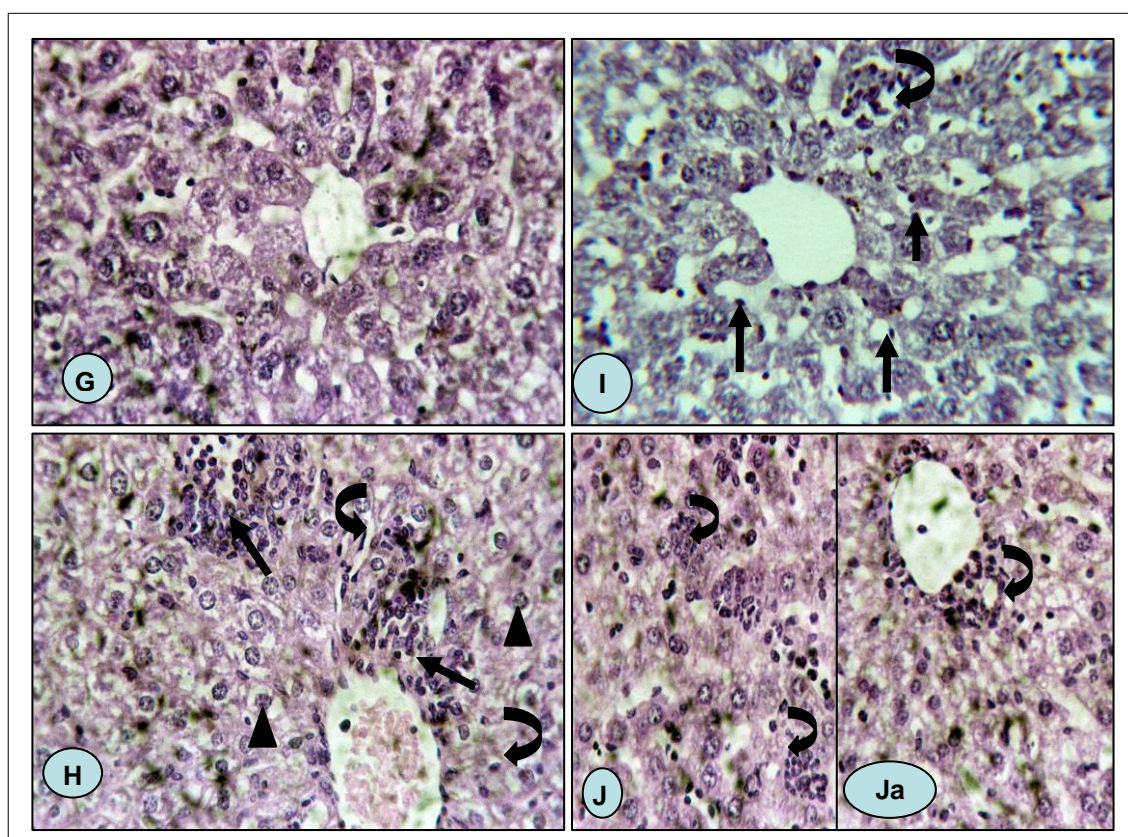


Fig.6 Photomicrographs of sections in liver of mice bearing SEC. **G**: Section in normal liver. **H**: Section in liver of mice bearing SEC **I**: Section in liver of mice bearing SEC treated by SA 24 hr ATE. **J&Ja**: Sections in liver of mice bearing SEC treated by SA 7 days ATE. (H&E. X400)

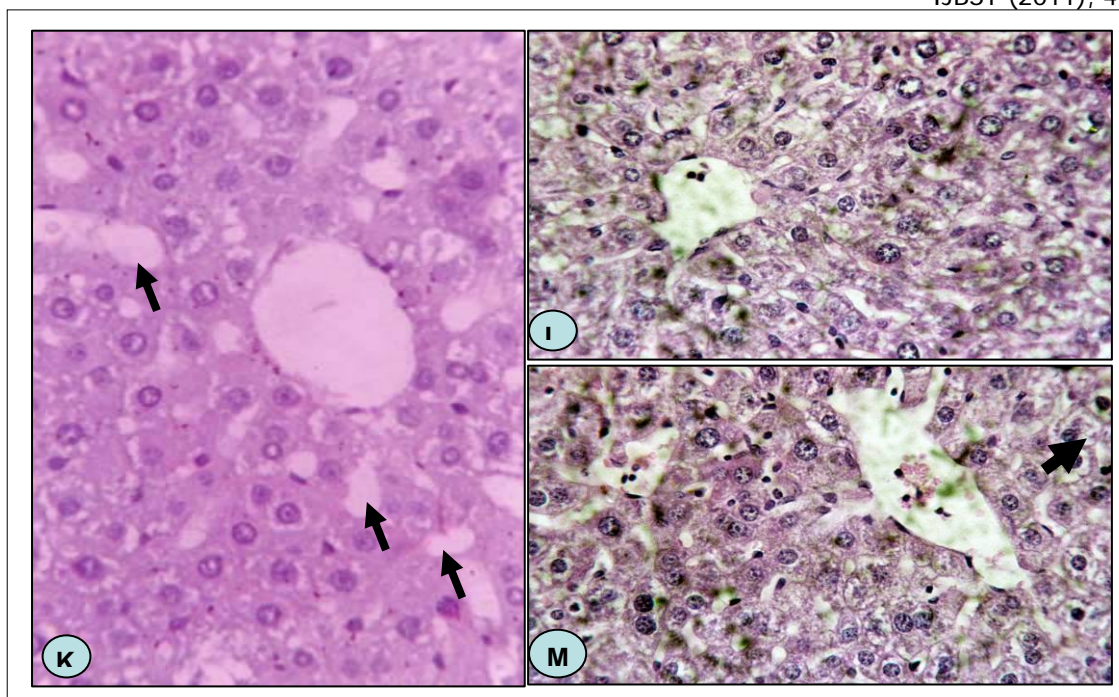


Fig.7 Photomicrographs of sections in liver of mice bearing SEC. **K:** Section in liver of mice bearing SEC exposed to  $\gamma$ - radiation7 days ATE. **L:** Section in liver of mice bearing SEC treated by SA 24 hr ATE and exposed to  $\gamma$ - radiation7 days ATE. **M:** Sections in liver of mice bearing SEC treated by SA and exposed to  $\gamma$ - radiation 7 days ATE. (H&E. X400).

## DISCUSSION

In recent years, there is an increasing awareness that certain naturally occurring compounds in plants and other sources, have protective effects against environmental mutagens/carcinogens and endogenous mutagens [15]. It was shown that plant like SE which is a sharp occurring in wild state in Egypt noticed to have several glycoside used in treatment of various colics and pains [7]. In the present study a pronounced delay in EC tumor volume was recorded when experimental animals gavages administrated with SA either 24 hr or 7 days ATI which is advantages with the finding of Alberto et al., [16] whom reported that SE contain 15-keto pregnane glycosides. The effect of these compounds on the VEGF-induced in Kaposi's sarcoma cell proliferation was tested and the results indicated that all the compounds reduced the cell proliferation in a dose dependent manner. Also Amr et al. [5] reported that hot water extract of SA significantly reduced Ehrlich ascites carcinoma cells induced tumor growth and delayed animal death by 29 days.

Also the exposure to  $\gamma$ - radiation 7 days ATI caused a continuous delay of tumor volume recorded duo to radiation oxidative stress induction which reflected by the enhanced levels of peroxidative damage, DNA fragmentation, LDH activity and nitric oxide levels [4]. A benefit of ionizing radiation as a therapeutic tool is the possibility to apply it locoregionally thereby preventing systemic toxicity. However like chemotherapeutic agents ionizing radiation does not

affect all target cells, which can lead to severe side effects in the surrounding tissue after the therapy. In addition there are large numbers of human malignant tumor cells that respond poorly to ionizing radiation. However, radiation dose to the tumor can not be increased as needed because of the normal tissue toxicity in the radiation field. Hence there is a need for chemical agents which upon contact with tumor cells increase their sensitivity to radiation thus minimizing large doses of radiation and also spare normal tissue from the combined toxic effects [17]. In the present study more pronounced delay in tumor volume recorded when experimental animals gavages administrated with SA combined with exposure to  $\gamma$ - radiation in which the antitumor synergistic effect between radiation exposure and SA supplementation were detected.

Induction of apoptosis in tumor cells, a form of physiological death in unwanted or dysfunctional cells, is an appealing therapeutic approach [18]. Treatment of female mice bearing Ehrlich carcinoma tumor 24 h. or 7 days ATI for 3 weeks with SA represented high and wide zones apoptotic cells in tumor tissue. The results reported here suggested that hot water extracts of SE may have an immuno-modulatory potential associated with the content of phenolics, including flavonoids stimulating antiproliferation of tumor cells and appears to involve apoptosis-induced cell loss also [5]. Also the exposure to 6 Gy of  $\gamma$ - radiation on the 7<sup>th</sup> day ATI predict sporadic apoptotic yellow cells in tumor tissue due to induction and repair of DNA

damage, cell cycle disturbances, programmed cell death, alterations in gene expression and signal transduction pathways [19, 20]. Induction and repair of DNA damage are central among the several molecular targets for modifying cellular radiation responses and most often correlated well with cell death [21, 22]. Also radiation exposure produces peroxidation on biological membranes. Peroxidation brings about changes in the structure and functions of tumor membranes [23]. This oxidative damage of membranes is also closely linked with radiation induced apoptosis [24].

Gavages treatment of EC bears animals by SE either 24 hr. or 7days ATI combined with  $\gamma$ -radiation exposure recorded high and wide zones apoptotic cells in tumor tissue which predict the synergistic effect of SE and  $\gamma$ -radiation exposure in resulting tumor regression.

The results of functional tests together with histological observations suggest that either  $\gamma$ -radiation exposures or Ehrlich tumour inoculation treatments leads to serious changes in histology of mice organ tissues [25]. The increased formation of lipid peroxides and associated reactive oxygen species leads to damage in membrane integrity and other pathological changes.

Lipid peroxidation, an autocatalytic free radical chain propagating reaction, is known to be associated with pathological conditions of a cell. Malondialdehyde (MDA), the end product of lipid peroxidation, was reported to be higher in cancer tissues than in nondiseased organ [26]. In the present study SA treatment either after 24 h. or 7 days of tumor inoculation predicts a non significant change in lipid peroxidation level of tumor tissue. However the exposure of the tumor bearing mice to 6 Gy  $\gamma$ -radiation predict a significant increase in lipid peroxides in tumor tissue context with the finding of Anjali et al., [4] whom reported that Radiation induced oxidative stress in Ehrlich solid tumor in mice.

The previous revealed that  $\gamma$ -radiation induced oxidative stress as reflected by the enhanced levels of peroxidative damage in Ehrlich solid tumor in mice [4] and SA was an immunomodulatory components containing phenolics, including flavonoids play a role in oxidation inhibition [5]. So the combined treatments of tumor bearing mice with SA and  $\gamma$ -radiation exposure resultant a non significant change in lipid peroxides in tumor tissue

In the event of generation of  $O_2^{\bullet-}$  in the liver as a distant organ, due to oxidative stress caused by tumor burden the levels of MDA were significantly increased

in EC bearing animals when compared with control animals [6]. SA treatment showed ameliorative effect in liver tissue was predicted due to antioxidant activity of SA [5]. Also exposure to 6 Gy  $\gamma$ -radiation predict a significant decrease in lipid peroxides in liver tissue compared to EC bearing mice which may be due to the radioresponse of antioxidant enzymes which seemed to be significantly different in the liver of tumor burdened mice compared to controls [6]. Also the effect was recorded when experimental animals gavages administrated with SA combined with exposure to  $\gamma$ -radiation.

A non significant change in GSH and CAT levels in tumor tissue were predicted after gavages administration of SA 24 hr. ATI or 7 days ATI either alone or combined with  $\gamma$ -radiation exposure predict a non significant change in GSH and CAT which explain the more pronounced delay in tumor volume. However  $\gamma$ -radiation exposure either alone or combined with SA administration predict some ameliorative effect in SOD level compared to control tumor tissue level context with the finding of [4].

Glutathione, a potent inhibitor of the neoplastic process, plays an important role in the endogenous anti-oxidant system. It is found in particularly high concentration in the liver and is known to have a key function in the protective process. Excessive production of free radicals resulted in oxidative stress, which leads to damage to macromolecules, for example, lipid peroxidation *in vivo* [27]. In our study a significant decline in GSH, CAT and SOD levels in the livers of tumor-bearing animals was observed compared to the normal control level. The decrease in SOD activity in EAC bearing mice may be due to loss of  $Mn^{2+}$  containing SOD activity in EAC cells and the loss of mitochondria, leading to a decrease in total SOD activity in the liver [28]. Also the inhibition of SOD and CAT activities may be a result of tumor growth [27].

Also the exposure of the tumor bearing animals to 6 Gy of  $\gamma$ -radiation predicts the same effect. Gavages treatment of EC bears animals by SE either 24 hr. or 7days ATI either or combined with  $\gamma$ -radiation exposure 7days ATI resultant a significant amelioration in GSH, CAT and SOD levels in livers of EC bearing animals.

Histologically Treatment of female mice bearing Ehrlich carcinoma tumor 24 h. 7 days ATI for 3 weeks with SA represented large area of apoptosis, hydropic degeneration and nuclear debris in tumor tissue section. Ariens et al., [29] reported that tissue toxicity usually manifests itself, especially in the histological preparation, in the form of cell degeneration

accompanied by formation of large vacuoles, accumulation of fat and tissue necrosis which predict the toxicity of SE on tumor tissue [5]. Exposure to 6 Gy of  $\gamma$ - radiation revealed highly degenerative effect great areas of apoptosis in addition of some pyknotic nuclei. The increased formation of lipid peroxides associated with reactive oxygen species leads to damage in membrane integrity and other pathological changes [25].

Due to the synergistic effect of SE and  $\gamma$ -radiation exposure in resulting tumor regression, tumors extirpated from animals treated with SA 24 h. or 7 days ATI for 3 weeks and exposed to 6 Gy of  $\gamma$ -radiation on the 7<sup>th</sup> day ATI extensive areas of apoptosis, presence of remnant tumor cells contained pyknotic nuclei and nuclear debris were recorded.

Liver of animals bearing EC showed alterations as Kupffer cells hyperplasia, intense ballooning degeneration of hepatocytes, great aggregation of inflammatory cells infiltration and Ehrlich tumor cells. This finding context with the finding of Samia and Fatma, [30]. Ehrlich tumor cells infiltration may be due to tumour cells proliferation and move to invade the internal organs [31]. Great aggregation of inflammatory cells may be due to disorganization of the cytoplasm [32], or more probably because of mitochondrial degeneration. ballooning degeneration of hepatocytes believed to be caused by lysosomal enzymes and hydration [33].

Treatment of the experimental animals with SA 24 h. ATI for 3 weeks exhibited a pronounced antitumor effect represented increase in Kupffer cells and decrease in amount of inflammatory cells. Increase in Kupffer cells increase their anti-tumor effect via increasing the production of NO, TNF $\alpha$  and IFN $\gamma$  and these cytotoxic molecules inhibit the growth of tumor by damaging cellular DNA and inducing apoptosis [34]. The presence of inflammatory suggests a possible relationship with tumor angiogenesis. Therefore the decrease in its number meanted the decrease in tumor progression [35].

Exposure of experimental animals bearing EC to 6 Gy of  $\gamma$ - radiation on the 7<sup>th</sup> day ATI the liver cells were filled with some cytoplasmic material and the tissue represent some vacuolisation. According to Mollendroff [36], vascular formation is a cellular defense mechanism against substances injurious to cells. In such a case, these substances were segregated in the vacuoles and were thus prevented from interfering with cellular metabolism.

Normal appearance in liver tissue was detected when female mouse bearing Ehrlich carcinoma gavages with SA 24 h. ATI for 3 weeks and exposed to 6 Gy of  $\gamma$ -

radiation on the 7<sup>th</sup> day ATI. While in the group treated with SA on the 7<sup>th</sup> day ATI and exposed to 6 Gy of  $\gamma$ -radiation, a well developed hepatic cord was detected in addition to presence of ballooning degeneration observed in some hepatocytes.

The available data from *in vitro* tests and *in vivo* animal toxicity studies indicate that SA (15 mg/kg body weight) has a protective action against radiation and/or tumor-induced toxicity as evidenced by the lowered tissue lipid peroxidation and elevated levels of the enzymes and non-enzymatic antioxidants in liver. Combined treatment of tumor with SA and radiation enhances oxidative stress and cytotoxicity in tumor cells.

SA protects normal cells against radiation damage. This may offer potential therapeutic benefit, which warrants clinical study for application in cancer radiotherapy. Further studies are required to examine the clinical use and exact mechanisms behind a possible protective effect of SA.

## REFERENCES

- [1] Gruddy, S.M., (1991) Recent Nutrition Research, implications for foods of the future. *Annals of Medicine*, 23: 187-193.
- [2] Benjamin HSL., Padma PT., Jeffrey MT. (1990) *Allium sativum*: (garlic) and cancer prevention. *International Journal of vitamin and Nutrition Research*, 10:937-948.
- [3] Thapliyal R., Deshpande SS., Maru GB. (2002) Mechanism(s) of turmeric-mediated protective effects against benzopyrene-derived adducts. *Cancer Letters*, 175:79-88. doi: 10.1016/S0304-3835(01)00675-00679.
- [4] Anjali A., Dharamainder C., Meenakshi U., Rath P.C. and Kale R.K. (2001) Radiation induced oxidative stress: I. Studies in Ehrlich solid tumor in mice. *Molecular and Cellular Biochemistry*, 223(1-2): 71-80.
- [5] Amr A. N., Ahmed M. AE., Khalid M. AE., David A. L., Alan C. and Hany A. ES. (2009) Anti-cancer and anti-oxidant activity of some Egyptian medicinal plants *Journal of Medicinal Plants Research*, 3(10):799-808.
- [6] Anjali A., Dhyani C. and Kale R.K. (2001) Radiation induced oxidative stress: II Studies in liver as a distant organ of tumor bearing mice *Molecular and Cellular Biochemistry* 224: 9-17.
- [7] Eltahir KEH., Geel AMMA., Mekkawi AG., Bashir AK., Mossa JS. and Khaled SA. (1987) Pharmacological action of the leaves of SA argel (Hyene): Spasmolytic and uterine relaxant activities. *Intl. J. Crud Drug Res.*, 25: 57-63.
- [8] Loewenthal H., Jahn, G. (1932) *Übertragungversuche mit carcinomatöser Mause-Ascitesflussigkeit und ihr*

- Verhalten gegen physikalische und Chemische Einwirkungen. *Ztschr F Krebsforsch*, 37:439, 1932.
- [9] Mamdooh G., Nariman K. B. E., Eman N., Lucilene T. (2008) *Saccharomyces cerevisiae*, the Baker's Yeast, suppresses the growth of Ehrlich carcinoma-bearing mice. *Cancer Immunol Immunother*, 57:581–592.
  - [10] Bank. H.L. (1988) Rapid assessment of islet viability with acridine orange and propidium iodide. *In Vitro*, 4: 266-273.
  - [11] Moron MS, Depierre JW, Mannervik B. (1979) Levels of glutathione, glutathione reductase and glutathione-s-transferase activities in rat lung and liver, *Biochim Biophys Acta*, 582: 67–78.
  - [12] Beuege JA., Aust SD. (1978) Microsomal lipid peroxidation. *Method Enzymol*, 30: 302–310.
  - [13] Kakkar P., Das B., Viswanathan PN. (1984) A modified spectrophotometric assay of superoxide dismutase. *Ind J Biochem Biophys*, 21:130–132.
  - [14] Maehly AC., Chance B. (1954) In: *Methods of Biochemical Analysis*. Vol. I, Glick D, editor. New York: Interscience, p. 357.
  - [15] Premkumar K., Abraham S. K., Santhiya S. T., Ramesh A. (2004). Protective effect of *Spirulina fusiformis* on chemical-induced genotoxicity in mice. *Fitotherapia* 75, 24-31.
  - [16] Alberto P., Angela P., Maria L. B., Francesca F., Ciro B., Arafa I. H., Cosimo P. and Sonia P. [2005] New unusual pregnane glycosides with antiproliferative activity from *Solenostemma argel*. *Steroids*, 70 (9): 594-603.
  - [17] Ganesh C. and Venkatasubbaiah A. K. (2005) Enhancement of Radiation Effect by *Aphanamixis polystachya* in mice transplanted with Ehrlich ascites carcinoma. *Biol. Pharm. Bull.*, 28(1): 69—77.
  - [18] Wyllie A. H. (1997) Apoptosis and carcinogenesis. *Eur. J. Cell Biol.*, 73: 189–197.
  - [19] Maity A., Kao GD., Muschel RJ., McKenna WG. (1997) Potential molecular targets for manipulating the radiation response. *Int J Radiat Oncol Biol Phys.*, 37:639-653.
  - [20] Bhattacharya RK. (2001) Signal transduction events in mammalian cells in response to ionizing radiation. *Ind J Exp Biol*, 39: 727-734.
  - [21] Iliakis G. (1991) The role of DNA double strand breaks in ionizing radiation induced killing of eukaryotic cells. *Bioassay*, 13: 641-648.
  - [22] Khanna KK., Jackson SP. (2001) DNA double strand breaks: signaling repair and the cancer connection. *Nature Genet*, 27:247-54.
  - [23] Leyko W. , Bartosz G. (1986) Membrane effects of ionizing radiation and hyperthermia. *Int J Radiat Biol* 49: 743–770.
  - [24] Ramakrishnan N., Kalinich JF., McClain DE. (1998) Radiation-induced apoptosis in lymphoid cells: Induction, prevention and molecular mechanisms. In: E.A. Bump, K. Malaker (eds). *Radioprotectors: Chemical, Biological and Clinical Perspectives*. CRC Press LLC, Florida, pp 253–273.
  - [25] El-Tahawy N.A., Hanafi N., and Said U.Z. (2009) Antitumor and Antioxidant Activities of Activin in Kidney Tissue of Mouse Bearing Murine Mammary Adenocarcinoma and Exposed to Gamma Radiation. *Egypt. J. Rad. Sci., Applic* 22(2): 427-443.
  - [26] Yagi K. (1987) Lipid peroxides and human diseases. *Chem. Phys. Lipids.*, 45:337–351.
  - [27] Sinclair AJ., Barnett AH., Lunie J. (1990) Free radical and auto-oxidant systems in health and disease. *Br. J. Hosp. Med.* 43: 334-344.
  - [28] Sun Y., Oberley LW., Elwell JH., Sierra Rivera E. (1989) Antioxidant enzyme activities in normal and transformed mice liver cells. *Int. J. Cancer*, 44:1028–1033.
  - [29] Ariens E.J., Simonis A.M., Offermeier J. (1976) *Introduction to general toxicology*. Academic Press, Inc. New York.
  - [30] Samia M. A., Fatma M Fouda (2009) Histological and histochemical study on the effect of Ehrlich ascites carcinoma on the liver and kidney of mice and the possible protective role of tetrodotoxin. *Egyptian Journal of Biology*, 11: 13-25.
  - [31] Chakraborty T., Bhuniya D., Chatterjee M, Rahaman M., Singha D., Chatterjee BN., Datta S., Rana A., Samanta K., Srivastawa S., Maitra S. K., Chatterjee M. (2007) *Acanthus ilicifolius* plant extract prevents DNA alterations in a transplantable Ehrlich ascites carcinoma- bearing murine model. *World Journal of Gastroenterology* 13 (48): 6538-6548.
  - [32] Hashimoto S., Koji T., Niu J, Kanematsu T., Nakane PK. (1995) Differential staining of DNA strand breaks in dying cells by non-radioactive in situ nick translation. *Archives of Histology and Cytology* 58: 161-170.
  - [33] Fukuda K, Kojiro M, Chiu Jen FU (1993) Demonstration of extensive chromatin cleavage in transplanted Morris hepatoma 7777 tissue: apoptosis or necrosis? *American Journal of Pathology* 142: 953-946.
  - [34] George G. C., Wan Y. L., Paul B.S. L., Ying S. C., Ernest C.W. C., Billy C.S. L., Isa K.Y. L., Janet F.Y. L., Albert K. C. (2002): Activation of kupffer cells inhibits tumor growth in a murine model system. *Int. J. Cancer*, 99: 713–720.
  - [35] Melchiorre C., Daniela F., Ada M. F., Maurizio S., Claudio T., Natale D'Al., Giuseppe M. (2005) Correlation between expression of cyclooxygenase-2 and the presence of inflammatory cells in human primary hepatocellular carcinoma: Possible role in tumor promotion and angiogenesis. *World J. Gastroenterol*, 11(30):4638-4643.
  - [36] Mollendroff (1973) Quoted from Bourne (1964) *Cytology and Cell Physiology* 3rd. edition, New York, Academic Press.